TRANSLATION NO. 921

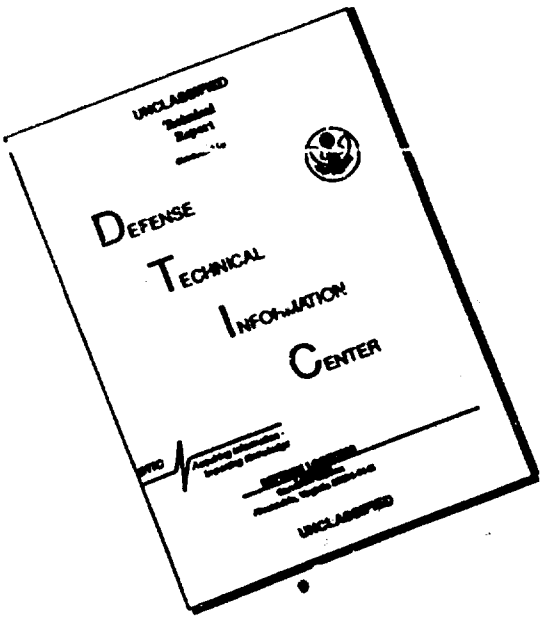
DATE: July 1968

DDC AVAILABILITY NOTICE

This document has been approved for public release and sale; its distribution is unlimited.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DISCLAIMER NOTICE



THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.

THE USE OF FLUORESCENT ANTIBODIES FOR THE ACCELERATED DETECTION OF DYSENTERY BACTERIA

[Following is a translation of an article by Candidate of Medical Sciences S.I. D'yakov, R.V. Kasatkina, Candidate of Medical Sciences V.M. Nikitin, and Z.V. Pestryakova from the Department of Microbiology of the Military Medicine Order of Lenin Academy imeni S.M.Kirov and the Sanitary Epidemiological Station (Leningrad) in the Russian-language periodical Gigiyena i Sanitariya (Hygiene and Sanitation), No 10, Moscow, 1962, pages 59-62. The article was submitted for publication on 29 January 1962.]

The existing methods of detecting pathogenic and sanitary-indicator microorganisms in milk, as in many other rapidly spoiling products, are long; there, the development of rapid and dependable methods of indicating pathogenic microbes in food products is an important task of sanitary bacteriology. In the present article data are given on the use of the immuno-fluorescent method for the rapid detection of dysentery pathogens in milk.

We prepared some extremely thin smears of milk. After drying the preparation we fixed it with Nikiforov's mixture for 10 to 15 minutes. In order to eliminate the background fluorescence, the smears were

given an additional treatment with benzene for 2 to 5 minutes.

In the work we used fluorescent conjugates of adsorbed Flexner's dysentery serum and its gamma globulin fraction which were marked by fluorescein isocyanate and fluorescein isothiocyanate (the fluorochromes were kindly presented to us by G.I. Mikhaylov to whom we are deeply grateful). For control staining we employed fluorescent conjugates of both normal and heterological (tularemia and typhoid) rabbit serums and their gamma globulin fractions. As an additional control we used the treatment with dysentery conjugates of preparations of samples of milk which knowingly did not contain dysentery bacteria. The preparations were stained for 10 to 20 minutes at 37 degrees Centigrade in a humid chamber. The smears were washed with a stream of tap water for 2 to 3 minutes; after drying they were examined under an ML-1 luminescent microscope using SZS- 7, FS-1, BS-8, and T-2N light filters. For the study of the general microflora of the milk and its quantitative evaluation, the smears were stained with a 1:1,000 solution of auramine for 1 to 2 minutes. After staining they were dipped in 70% alcohol for several seconds and then were carefully washed with water.

The use of the fluorescent antibodies method for the accelerated detection of dysentery bacteria in milk can satisfy the practical requirements in three basic respects: rapidity in obtaining an answer, sensitivity, and specificity. As a rule, 30 to 45 minutes were sufficient for the preparation, staining, and microscopic examination of the preparations. In

order to determine the sensitivity of the method, 5 to 10 milliliters of bottled milk were infected with various doses of Flexner's (Type C) dysentery bacteria. After 30 minutes of contact the samples were carefully mixed and smears were prepared from them which were stained with fluorescent conjugates and a solution of auramine. The smears which were stained with auramine showed a large amount of brightly fluorescent yeast cells, bacilliform and spherical cells, etc. In contrast to this, the preparations which had been treated with fluorescent dysentery conjugates, depending on the concentration of dysentery bacteria which were introduced into the sample, showed the presence of various amounts of bacilliform microbe cells which were intensely fluorescent with a yellow-green light and which had even more intensely fluorescent edges. The results obtained are given in the table.

Summary indices of the sensitivity of the method of fluorescent antibodies in detecting dysentery bacteria in artificially infected samples of milk

Concentration of dysentery bacteria in 1 ml of test milk	Quantity of fluorescent cells in the field of vision										
		centrifuging			flotation				culturing in Maryin's bouillon		
	direct microscopy	creamy film	liquid above the precipitate	precipitate	in 20 minutes	in 2 hours	in 24 hours	after centrifuging	in 6 hours	in 24 hours	after 6 hours followed by centrifuging
100,000,000	10-20	tens	0-1	tens	tens	tens	tens	tens	up to 5-10	10-20	tens
10,000,000	1-5	10-20	-	tens	3-10	tens	tens	tens	1-3	10-20	tens
1,000,000	0-1	up to 10	-	up to 20	0-2	up to 10	up to 20	tens	0-1	up to 10	tens
100,000	0-1	1-2	-	1-5	-	1-5	up to 10	up to 10	-	up to 10	10-20
10,000	-	0-1	-	1-2	-	0-1	2-3	2-3	-	up to 3-5	up to 10
1,000	-	0-1	-	1	-	1-2	1-2	1-2	-	up to 3-5	1-2-3
100	-	-	-	-	-	-	-	-	-	-	-

In order to increase the sensitivity of the fluorescent antibodies method we employed methods of concentrating the bacteria in the milk: centrifuging, flotation, and brief culturing in nutrient media, etc. The infected samples of milk were centrifuged in volumes of 5 to 10 milliliters at 2,500 to 3,500 revolutions per minute for 5 to 60 minutes. The smears were made from the upper creamy film, the clear liquid above the precipitate, and the whitish precipitate. For flotation, 0.3 to 0.5 milliliters of xylol were added to 9 milliliters of a test sample of milk, after which the test tubes with rubber stoppers were agitated for 10 to 15 minutes in an agitating apparatus. For culturing, 0.2 to 1 milliliter volumes of milk were seeded in a semi-liquid medium of Ploskirev's medium, Martin's bouillon and mannitol (0.5%). Muller's fluid and the Kessler-Svenarton medium were also tested. The remaining milk in a volume of 5 milliliters was put in a thermostat at 37 degrees Centigrade. Smears were prepared after 2,4,6,12, and 24 hours of incubation in the thermostat. The summary indices of the tested methods of increasing the sensitivity are given in the table.

The indices which were obtained indicate that the employment of additional steps which make it possible to concentrate the microbe cells increases the sensitivity of the fluorescent antibodies method to the extent of obtaining positive results for the presence of from 1,000 to 10,000 microbe cells of dysentery bacteria in 1 milliliter of tested milk.

The matter of the specificity of any method of

diagnosis in the final analysis actually determines its practical value. First of all we studied the intensity of the specific staining of the dysentery bacteria in relation to their being in artificially contaminated milk. With this aim samples of raw bottled milk were infected with 50 million microbe bodies per milliliter and were kept for 10 days at 2,4,18-22, and 37 degrees Centigrade. Smears were prepared daily. At the same time we conducted seedings in differential-diagnostic and elective batches of Endo's and Ploskirev's cultural mediums. The results of the tests showed that the cells of the dysentery bacteria over the entire observation period did not lose their ability to be stained specifically by fluorescent antibodies; however, the intensity of the luminescence of the cells and the quantity of fluorescent cells in the field of view of the microscope were lowered somewhat. It is interesting to note that beginning from the third day of the incubation of samples of infected milk in the thermostat at 37 degrees Centigrade and at room temperature, the number of colonies of dysentery bacteria which grew in the dishes gradually decreased and, beginning with the eighth day, dysentery microbes were not sown at all. The positive finding in the indicated samples of fluorescent cells demonstrates that the fluorescent-immunological method exposed the unviable cells of dysentery bacteria.

Our basic attention in studying the specificity of the fluorescent antibodies method was directed at investigating commercially-produced milk. In all we investigated 181 samples of such milk. Upon receiving

the samples at the laboratory we immediately prepared smears directly from the milk, from the creamy film (after 5 to 10 minutes of centrifuging 5 to 10 milliliters of milk at 3,000 revolutions per minute), and also from the precipitate after 6 hours of culturing in Martin's bouillon and centrifuging the seedings. The preparation was carefully examined for three minutes under a fluorescent microscope. In recording the data we devoted our attention to the morphology of the fluorescent formations, the intensity and color of their fluorescence, the nature of the staining, and the quantity of fluorescent cells in the field of vision or in the preparation. In evaluating the fluorescent images the results were compared with the data from the control preparations which were treated with normal and heterogeneous fluorescent serums. Parallel with this, each sample of milk was tested bacteriologically for coli titer, microbe number, and also the presence of dysentery and typhoid-paratyphoid bacteria. In addition, the colonies which grew in Sense culture mediums were tested on Bacillus coli which had been positively agglutinated by Flexner's dysentery bacteria.

The results of the observations which were conducted were as follows. In 99 samples (54.7%) not a single fluorescent cell was discovered in the luminescent microscopy of all three series of samples. Bacteriological tests for the presence of dysentery bacteria also gave negative results. In five samples strains of Bacillus coli which were agglutinated by Flexner's dysentery serum were isolated. In 9 samples (5%) intensively fluorescent cells with a characteristic

staining structures were detected after treatment of the preparations with normal fluorescent conjugates.

In 23 samples of milk (12.7%) fluorescence of the microbe cells was observed when the smears had been treated with both dysentery and normal fluorescent serums. This indicates that the observed fluorescence is a non-specific staining of the microbe cells. With a bacteriological test one strain of Morgan's bacillus and 6 strains of Bacillus coli which were agglutinated by Flexner's serum were isolated. Thus, summarizing the data from the testing of 131 samples of milk (72.4%), we can consider that they all belonged to the negative group. According to the data from luminescent microscopy, 28 samples of milk (15.4%) should be listed under the weakly positive group. All these samples were characterized by the fact that upon staining with dysentery serum, only individual specifically fluorescent cells were detected (1 to 4 for all of the smears). From these smears, 7 strains of Bacillus coli were isolated which were agglutinated by dysentery serum. Dysentery bacteria were not discovered in these samples. With a weakly positive response in a given series of samples we oriented upon the individual nature of the findings of fluorescent cells in the entire preparation and on the absence of an increase in the number of fluorescent cells in the preparations which were prepared from Martin's bouillon after 6 hours of culturing.

Finally, the remaining 22 samples of milk (12.2%), according to the data of the fluorescent antibodies method, can be assigned to the group with a

clearly positive response. However, it should be emphasized that not in a single case was it possible to detect dysentery bacteria in these samples using a bacteriological test. In 21 samples of milk we isolated strains of Bacillus coli which contained antigens which were common with Flexner's dysentery bacteria.

Thus, despite the negative results of the bacteriological testing of 181 samples of commercial milk, 22 of the samples, according to the luminescence analysis data, were positive. Obviously further research is required in order to evaluate the positive results of the fluorescent method.

Of some interest is the material from the detailed study of 62 parastrains which we isolated from the milk. After the isolation of the pure cultures and their brief laboratory storage, we noticed a sharp lowering of both the ability of the cultures to be agglutinated by Flexner's dysentery serum and the intensity of the luminescence of the microbe cells which had been stained by fluorescent dysentery conjugates. Thus after 2 or 3 resowings the microbe cells of 46 parastrains fluoresced to ++ and only individual cells of some of these strains have a brighter fluorescence of up to +++. Only 4 strains fluoresced sufficiently brightly (+++), while individual cells fluoresced up to ++++; 12 strains completely lost the ability to fluoresce. Most of the strains were characterized by a diffused luminescence of the entire body of the microbe cell of a weak or average intensity (+ and ++). As a rule, brightly fluorescent edges were absent in all parastrains, although there were four exceptions.

Only some small grain-like sectors on the surfaces of some microbe cells fluoresced more brightly, sometimes creating the impression of bipolar luminescence. There were particularly sharp manifestations of uneven staining of the cells in the preparations.

The peculiarities which were noted in the fluorescent staining of the cells of parastrains in pure cultures made it possible to distinguish with sufficient accuracy between the majority of them and Flexner's dysentery bacteria.

BIBLIOGRAPHY

Yefimova, K.V., Laboratornoye delo (Laboratory Work), 1959, No 3, page 45.

Kaufman, F., Semeystvo kishechnykh bakteriy (The Family of Intestinal Bacteria), Moscow, 1959.

5570
CSO: 8256-D